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Estimation of HIV-1 DNA Level Interfering with Reliability of HIV-1 RNA Quantification Performed on Dried Blood Spots Collected from Successfully Treated Patients

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The impact of HIV-1 DNA coamplification during HIV-1 RNA quantification on dried blood spots (DBS) was explored. False-positive HIV RNA detection (22/62, 35%) was associated with high HIV-1 DNA levels. Specificity of HIV-1 RNA assays on DBS should be evaluated following manufacturer protocols on samples with HIV-1 DNA levels of ≥1,000 copies/10^6 peripheral blood mononuclear cells.

Using plasma specimens for viral load (VL) testing is the preferred approach to detect treatment failure in HIV-1-infected people on antiretroviral therapy (ART) (1). However, widespread implementation of VL monitoring remains challenging in resource-limited settings, especially in remote areas, because of insufficient laboratory infrastructures and a lack of human resources. VL testing on dried blood spot (DBS) specimens can be an alternative to testing in plasma to overcome logistical, infrastructural, or operational barriers.

The use of DBS offers the advantages of a stable and easy-to-collect specimen and improved shipment with minimal infectious risk for testing with most available platforms in central laboratories. Studies have shown that DBS specimens may be useful for early diagnosis of pediatric HIV-1 infection, treatment monitoring, external quality assessment programs, and resistance testing (2–4). HIV-1 RNA testing on whole blood collected on DBS is highly effective in determining viral failure at the clinical threshold of 5,000 copies/ml and may be used effectively at a threshold of 1,000 copies/ml, although a suboptimal sensitivity was reported for this level (5).

Standardized protocols, including a step for blood elution from filter paper, are needed for successful implementation of HIV-1 load testing using DBS. Advancements were made in the last 5 years with DBS protocols for HIV load provided by bioMérieux, Roche, Abbott, Biocentric, and Siemens (6). The bioMérieux NucliSENS assay is currently Conformité Européenne In Vitro Diagnostics (CE-IVD) approved for DBS specimens. Other manufacturers of HIV RNA nucleic acid tests need to pursue regulatory approval for *in vitro* diagnostics on DBS (6, 7).

One of the current limitations of measuring HIV-1 load with DBS is interference of HIV-1 DNA coamplified during HIV-1 RNA quantification by reverse transcription-PCR, notably, among samples collected from patients who were successfully treated with ART (7–11). Here, we determined the HIV-1 DNA level that interfered with the reliability of HIV-1 RNA quantification on DBS without using a DNase pretreatment step or a specific RNA extraction method. It enabled the establishment of an HIV-1 DNA threshold with a high risk of false-positive HIV-1 RNA results on DBS specimens.

We randomly collected venous blood samples from 69 HIV-1-infected outpatients after receiving informed consent and obtaining the approval of local ethics committees from Burkina Faso and France (004-2012/CE-CM and ID RCB 2011-A01566-35, respectively). All patients were treated with ART for at least 6 months. Fifty microliters of blood collected on EDTA tubes was spotted onto five 12-mm circles on a Whatman 903 filter paper card (Whatman GmbH, Dassel, Germany) and air dried for 1 day prior to storage at −20°C. For elution, two entire 12-mm spots were placed in 2.0 ml of lysis buffer under constant shaking for 1 h as previously described (2). DBS HIV-1 RNA was then extracted by the NucliSENS miniMag extraction system (bioMérieux, Marcy l'Etoile, France) or by the large-volume nucleic acid extraction kit using the Arrow extraction system (Biocentric, Bandol, France). Plasma HIV-1 RNA was extracted from 200 μl of matched plasma with the QIAamp viral RNA minikit (Qiagen, Courtaboeuf, France). HIV-1 DNA was extracted from matched whole blood with the QIAamp DNA minikit (Qiagen). Plasma and DBS HIV-1 RNA levels were quantified using the Generic HIV load assay (Biocentric) with a lower detection limit of 300 copies/ml in plasma and an estimated ~2,000 copies/ml on DBS specimens (2, 3). HIV-1 DNA quantitation was performed with the Generic cell

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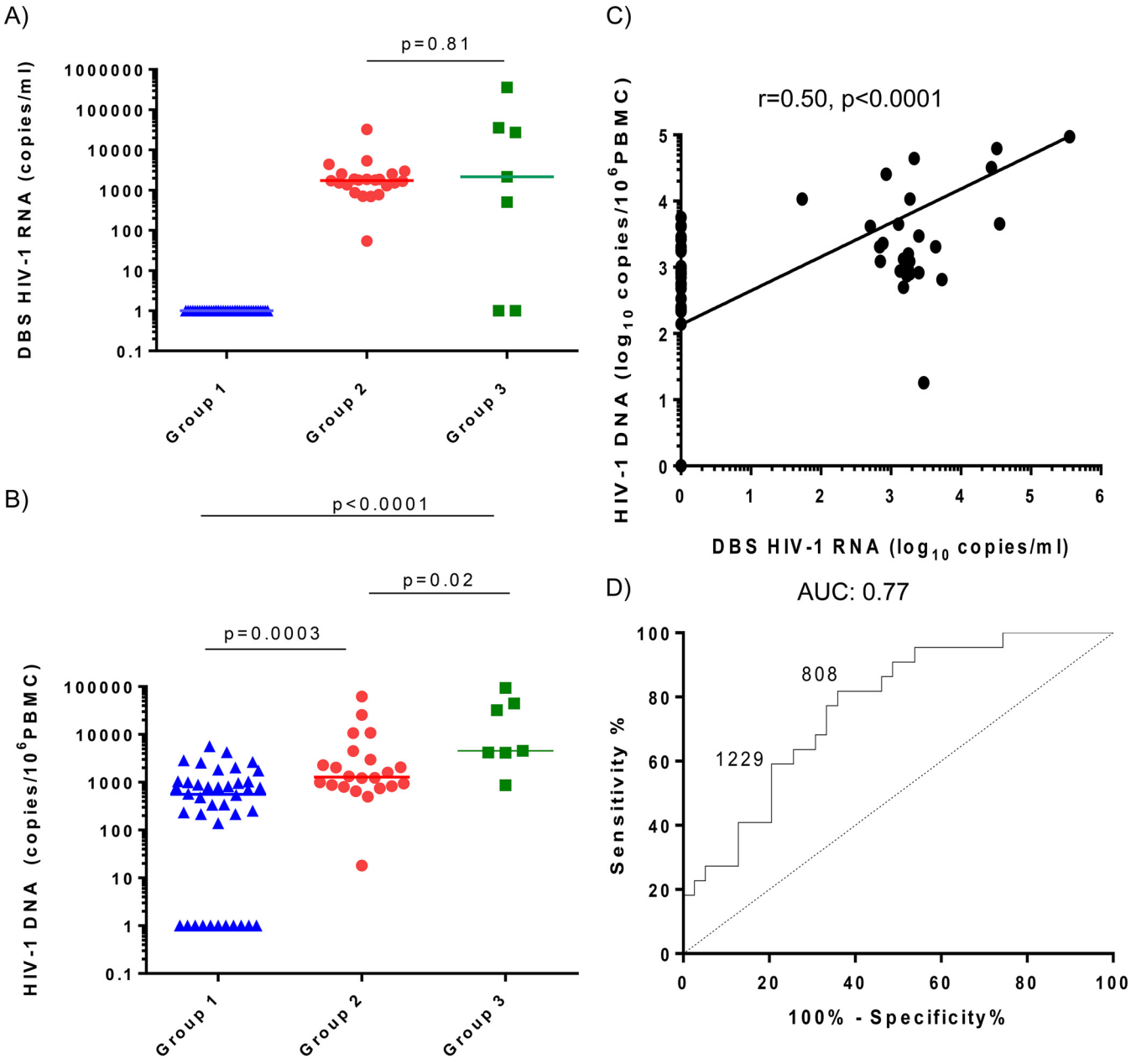

FIG 1 HIV-1 RNA quantification on DBS according to HIV-1 DNA levels. Subjects were classified in the three following categories: group 1 ($n = 40$), HIV-1 RNA plasma$^-$/DBS$^-$; group 2 ($n = 22$), HIV-1 RNA plasma$^-$/DBS$^+$; group 3 ($n = 7$), HIV-1 RNA plasma$^+$/DBS$^+$ or DBS$^-$. (A) HIV-1 RNA levels on DBS according to HIV-1 RNA results in plasma. (B) HIV-1 DNA levels in whole blood according to HIV-1 RNA results in plasma and DBS. (C) Correlation between HIV-1 RNA load results on DBS and HIV-1 DNA levels in whole blood. (D) ROC curve displaying sensitivity versus specificity of HIV-1 RNA quantification on DBS for the prediction of the risk of false-positive VL results with DBS specimens. Thresholds at 1,229 and 880 HIV-1 DNA copies/10^6 PBMC and area under the curve (AUC) are indicated. *r*, Spearman correlation coefficient; *p*, Mann-Whitney test.

HIV DNA assay (Biocentric), with a detection limit of 5 copies per PCR. HIV-1 DNA results were expressed as copies per million peripheral blood mononuclear cells (PBMC) using the results of the blood formula.

Subjects were assigned to one of three groups: group 1, undetectable for HIV-1 RNA in plasma and DBS specimens; group 2, undetectable for HIV-1 RNA in plasma but positive for HIV-1 RNA on DBS; and group 3, detectable for HIV-1 RNA in plasma regardless of HIV-1 RNA results on DBS.

From a total of 69 patients, the plasma samples of 62 patients (90%) were negative for HIV-1 RNA. As shown in Fig. 1A, among the 62 aviremic patients, 40 were negative for HIV-1 RNA on DBS specimens (group 1), whereas 22 (35%) had false-positive results with testing on DBS (group 2). HIV-1 DNA levels were higher in group 2 patients than in group 1 patients (Mann-Whitney test, $P = 0.0003$) (Fig. 1B).

HIV-1 RNA was detected in the plasma of seven patients (median, 2,156 copies/ml; interquartile range, 1,198 to 23,709 copies/

ml) (group 3). Five of them (71%) were found to be positive for HIV-1 RNA on DBS, whereas the two remaining patients with negative DBS results showed low plasma viremia (2,156 and 115 copies/ml) (Fig. 1A).

As shown in Fig. 1C, a significant correlation was observed between HIV-1 DNA levels and HIV-1 RNA load on DBS (Spearman test, $r = 0.50$; $P < 0.0001$). Based on a receiver operating characteristic (ROC) curve analysis, we estimated that the contamination of HIV-1 RNA load on DBS by HIV-1 DNA was noticeable from 800 to 1,200 DNA copies/10^6 PBMC (Fig. 1D).

Our results confirmed that cell-associated HIV-1 DNA was released in DBS elution liquid in a quantitative manner and consequently was amplified when levels of HIV-1 DNA were high, often leading to detectable HIV-1 RNA testing in patients treated successfully with AIDS-associated retrovirus. The threshold beyond which HIV-1 DNA frequently contaminates RNA quantification was estimated to be ~800 to 1,000 HIV-1 DNA copies/10^6 PBMC.

Current DBS protocols for commercially available VL tests should be challenged for their capacity to accurately identify successfully treated patients despite HIV-1 DNA levels of ≥1,000 copies/10^6 PBMC and treatment failure at a threshold of 1,000 HIV-1 RNA copies/ml. Reaching this balance is key for expanded DBS use during ART monitoring.

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